Radically New Research

The role of free radical formation in the development of various diseases and disorders has received extensive media attention in recent years. In turn, the public's fascination with this topic has grown, and manufacturers have been quick to tout products such as vitamins and herbal supplements as "free radical scavengers" and antioxidants that they claim prevent disease by halting free radical damage.

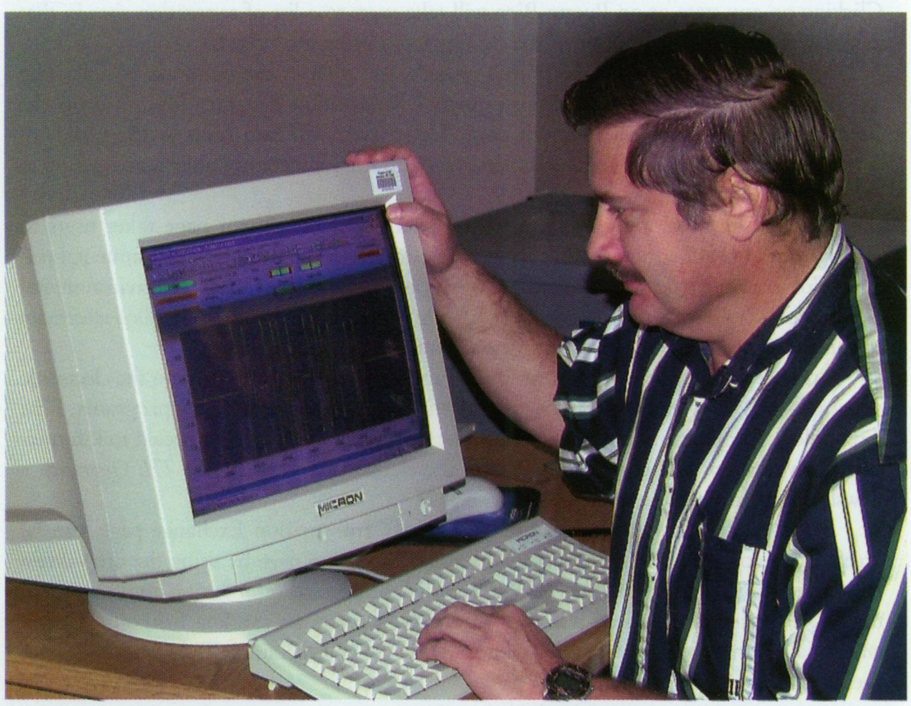
While some information about these processes comes from reliable sources, researchers are skeptical about the ability of many consumer products to actually prevent disease. The issue is controversial, and many questions still remain due to unreliable testing methods as well as conflicting test results.

In the wake of this public frenzy, NIEHS researchers have continued to engage in careful scientific studies to further clarify the role of free radicals. They have made significant strides in the areas of free radical formation and oxidative stress, or the body's response to the reaction of free radicals with oxygen, a response that can lead to disease and death.

Free radicals are any reactive organic or inorganic molecules with one or more unpaired electrons. Due to their highly reactive nature, they are difficult to detect. Free radicals are commonly formed in the body as a result of metabolic processes. Many are formed through everyday metabolic processes of common substances such as oxygen, which is reduced to the free radical superoxide. The body has equipped itself to eliminate free radicals; for instance, the enzyme superoxide dismutase protects oxygen-metabolizing cells against the superoxide free radical. However, free radicals that are not destroyed can be toxic because of their propensity to react with biological molecules such as lipids, proteins, and DNA. In particular, the chemical reactions of free radical metabolites of toxic chemicals and drugs can have toxicological consequences that may cause cellular damage and death.

Free radicals are thought to play a major role in the normal aging process. Free radicals are also strongly associated with many, if not most, toxicological processes. In fact, many chemicals are toxic because they are metabolized to free radicals. For instance, both carbon tetrachloride, a liver toxicant, and paraquat, a pulmonary toxicant, act through their free radical metabolites. Furthermore, many health conditions are thought to be associated with free radical damage, including heart disease, atherosclerosis, and arthritis.

While it is generally accepted that free radicals play a major role in toxicology and disease development, much remains to be discovered about that role. The first step in deciphering the role of free radicals is detecting and identifying them in the body. Currently, scientists have two approaches to this—directly detecting free radicals through a process called electron spin resonance (ESR) and using analytical "footprints," or indicators, that indicate that free radical activity has taken place.



Spin doctor. Research chemist Ron Mason analyzes electron spin resonance data to determine the presence of free radicals.

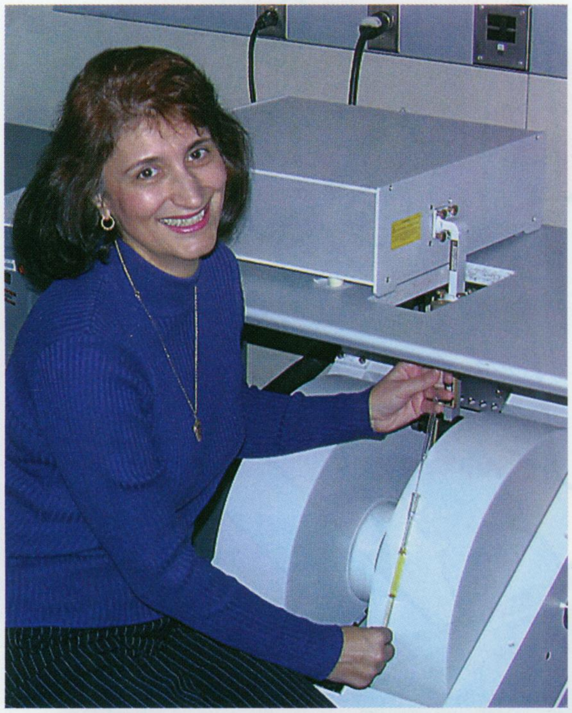
ESR

ESR, a spectroscopic technique that detects the unpaired electron present in a free radical, is the only general means of directly detecting free radicals. A free radical yields an ESR spectrum that can be used in its identification. While many free radicals are so reactive that they can not be detected by ESR, these free radicals can be stabilized by combining them with other molecules that can then be detected through ESR in a process known as "spin trapping."

Ron Mason, a research chemist in the Free Radical Metabolism Section of the Laboratory of Pharmacology and Chemistry, has pioneered the application of ESR to biological, pharmacological, and toxicological problems *in vivo*. He says it is critical that the experiments be conducted *in vivo* because unless free radical metabolites can be demonstrated with an animal model, there will continue to be some question as to their actual existence in biology. So far, Mason's group has demonstrated the formation of free radicals from rancid unsaturated fatty acids, established the role of hydroxyl radicals in iron and copper toxicity, and implicated the involvement of an ethanol-derived free radical in alcohol-induced cirrhosis of the liver. The group has also been active in examining metal-mediated free radical generation. High exposures to metals can result in toxicity, and researchers believe that cellular injury from toxic metals may result from metal-induced formation of free radicals.

Mason's group collaborated with the EPA to examine exposures to air pollution particles, which have been associated with increased human morbidity and mortality, although the mechanism by which this results is unknown. This work investigated the hypothesis that metals in pollution particles may initiate the generation of free radicals. In the study, rats were exposed to oil fly ash, which is an emission source air pollution particle. Using ESR and spin trapping, the researchers examined lung tissue and determined that free radicals had formed. They found the same results when they exposed a second group of rats to either vanadium or a mixture of vanadium, nickel, and iron sulfates such as occurs in air pollution particles. These results suggest that free radical generation is associated with the metals in the oil fly ash. The findings were reported in the October 1997 issue of *Chemical Research in Toxicology*.

While the toxicological implications of these findings are unknown, simply identi-



A stressful situation. Visiting scientist Maria Kadiiska is coordinating the evaluation of possible biomarkers of oxidative stress.

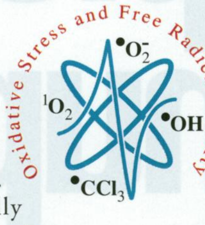
fying and detecting the presence of free radicals may be a significant step toward verifying the toxic components of air pollution particles, says Mason.

Another area the group has been studying is the production of nitric oxide (NO) in the metabolism of toxic chemicals and drugs. NO is an unusual free radical in that it is not highly reactive. It is commonly produced in metabolic processes and is usually beneficial, although overproduction of NO can have harmful effects.

The group has been studying the production of NO in the metabolism of the drug hydroxyurea, an anticancer agent that is also used to treat sickle cell anemia. In 1997, they determined that NO is produced when hydroxyurea is metabolized in rats, and they postulated that NO is also produced in humans who take the drug.

Recently, Richard Glover, a visiting fellow at the NIEHS, completed a study in humans with sickle cell anemia that was the first to indicate that hydroxyurea is metabolized to NO. Using ESR, the group detected nitrosyl hemoglobin within 30 minutes of oral administration of hydroxyurea to humans. Nitrosyl hemoglobin is NO bound to hemoglobin; thus is the presence of NO demonstrated. Because NO has been found to play a role in vasodilation (dilation of the arteries), the researchers suggest that the NO metabolite may be responsible for easing the pain of sickle cell anemia crises. This finding is significant both in that NO was directly detected in the blood of a human for the first time, and because it possibly sheds light on the role of NO in the drug's efficacy, says Mason.

Oxidative Stress Faculty



Markers of oxidative stress indicate the presence of free radicals in the body. It has been generally agreed among scientists who investigate free radicals that there is a need for validating sensitive and specific biomarkers for oxidative damage in rodents, nonhuman primates, and humans resulting from multiple types of oxidative insults. The NIEHS has taken the lead in coordinating the first comprehensive comparative study for determining which of the available biomarkers for oxidative stress are the most sensitive and selective. The study, called the Biomarkers of Oxidative Stress Study, was initiated by Carl Barrett, scientific director of the NIEHS, and is being organized and coordinated by Maria Kadiiska, a visiting scientist in the Free Radical Metabolism Section.

Researchers in 4 NIEHS laboratories and 12 outside laboratories are evaluating a battery of more than 25 assays as possible biomarkers of oxidative stress, using four models of oxidative stress: carbon tetrachloride poisoning, iron overload, ozone exposure, and paraquat poisoning. More than 2,500 samples of biological specimens are being evaluated. So far, Kadiiska says, two assays have emerged as the most promising for being truly indicative of oxidative stress—the measurement of isoprostanes and of protein carbonyls.

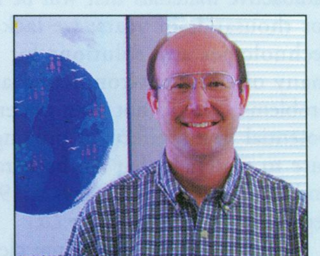
In principle, many free radicals can induce isoprostane generation. Isoprostanes are nonenzymatic prostaglandin-like products that result when free radicals catalyze the peroxidation of arachidonic acid. Their quantification appears to be a useful marker of oxidative stress such as the free radical oxidation of fats in the blood.

Protein oxidation products and carbonyl derivatives of proteins may result from oxidative modifications of amino acid side chains, reactive oxygen-mediated peptide cleavage, and reactions with lipid and carbohydrate oxidation products. The presence of carbonyl groups in proteins appears to indicate that the proteins have been subjected to oxidative free radical damage. Research has associated an increase in protein carbonyl content of tissues with several disorders, including rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, and atherosclerosis.

The difficulty of measuring highly reactive free radicals by ESR or biomarkers in cell culture and humans is particularly challenging. But the importance of free radicals in biology, toxicology, and disease means many scientists will continue to pursue these goals.

Brandy E. Fisher

Stokes Recognized



William S. Stokes, associate director for animal and alternative resources at the NIEHS, has been recognized by The Humane Society of the United States under its Russell and Burch Awards Program. The program honors scientists who work to advance alternatives to the use of animals in research, testing, and education. Stokes was lauded for his ongoing work as director of the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods, and as cochair of the Interagency Coordinating Committee on the Validation of Alternative Methods, which was established to review and implement alternatives to the use of animals in toxicity testing.

At the awards ceremony held 2 November 1998 in Washington, DC, Stokes received a framed certificate of recognition and a signed copy of the 1959 book *The Principles of Human Experimental Technique* by William Russell and Rex Burch. This book first introduced the concepts of replacement, reduction, and refinement, also known as the three Rs of laboratory animal welfare.